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Gigantism in tadpoles and color anomalies in European green toad *Bufotes viridis* from Poland

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Abstract

Some new cases of anomalies in wild *Bufotes viridis* from Western Poland are presented together with a discussion of their potential causes and a comparison with similar cases from the literature. The anomalies in question are color aberrations (pre- and post-metamorphic) and developmental pathologies (pre–metamorphic). The color anomalies include an ochre patch in a juvenile, leucism and albinism in both tadpoles and juvenile individuals. Developmental pathologies described in this paper include gigantism, edema, curvature of tail, and asymmetric bodies.

Key Words

aberration, Bufonidae, leucism, pathologies

Aberrant amphibian individuals can be found both in large (due to mere populations' size) and small populations. The latter is partly due to genetic drift (Hitchings and Beebee 1997) and, especially in the case of populations that are genetic isolates (like many urban amphibian populations), inbreeding (Hitchings and Beebee 1997; Vershinin 2004; Weyrauch and Grubb 2006). Weakened predation pressures in urban habitats might hypothetically also contribute to a higher prevalence of aberrant individuals in such places due to them usually being of lower fitness, hence benefiting from the scarcity of predators (Strachinis and Tsarouhas 2021). Atypically colored amphibians, usually having other co-occurring anomalies and looking less cryptic, show a lower survival rate than normally colored ones (Henle et al. 2017a); however, it seems that they are now quite frequently reported and attract general interest (Jablonski et al. 2014; Henle et al. 2017a; Kolenda et al. 2017). To date, several color anomalies have been reported in the European green toads *Bufotes viridis* (Laurenti, 1768), including albinism, axanthism, erythrism, leucism, abnormal ochre patches and retinal depigmentation (Flindt 1985; Andrä 2011; Jablonski et al. 2014; Henle et al. 2017c; Lunghi et al. 2017; Strachinis and Tsarouhas 2021). Herein, we present several rare or previously unreported color anomalies in *B. viridis* from Poland.

On 4 August 2017 at about 20:00 we found and photographed a juvenile individual of *B. viridis* with an ochre patch extending from the middle of the parotid glands onto the head (Fig. 1A, B). The observation was made in Tarnowo Podgórne, near man–made water reservoirs [52°27'53"N, 16°38'38"E], where dozens of freshly metamorphosed conspecifics with typical coloration were present. Unfortunately, the individual was in a closed and fenced area, so it was impossible to catch it for a more detailed inspection. As far as we observed, it did not behave differently from typically colored conspecifics.

Divergent patterns and colorations other than melanism and albinism can be caused by parasites,

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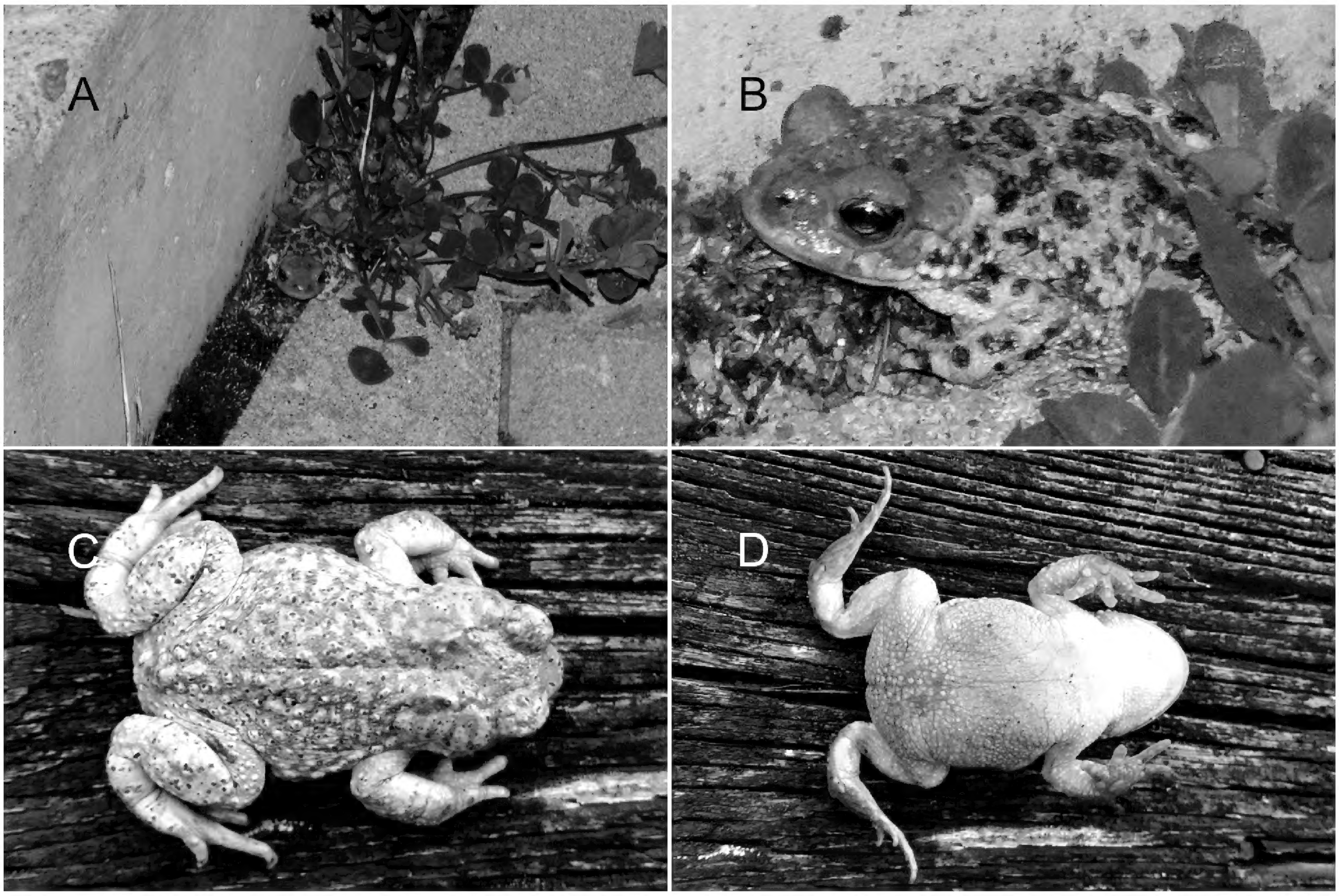

Figure 1. Aberrant juveniles of *B. viridis* from Poland. **A, B.** Juvenile with an ochre patch; **C, D.** Juvenile leucistic individual.

diseases, chemicals, inbreeding, radioactivity, or other mutagenic factors (Henle et al. 2017c). To date, *B. viridis* individuals with abnormal yellow/ochre patches were found in 1980 at a quarry in Roßwag, Germany (Henle et al. 2017c). Those individuals had yellow patches of various sizes either on the flanks or on the dorsum (see fig. 13 in Henle et al. (2017c) and fig. 32 in Henle et al. (2017a)). Henle et al. (2017c) crossed one of them with a normally colored individual, and 25% of the offspring developed ochre patch/patches, which indicates that they were heritable. The authors also speculated that the mutation was most likely caused by irradiation from radioactive contaminants. In our case, a sewage treatment plant located ca 200 m away and/or a waste pond for thermal spring water located ca 50 m away could have been the sources of mutagenic factors.

Atypical yellow dorsal spots dubbed as partial flavism were observed in the yellow–bellied toad *Bombina variegata* (Linnaeus, 1758) and in the spadefoot toad *Pelobates fuscus* (Laurenti, 1768) in two localities in Poland (Kolenda et al. 2017). In *B. variegata* yellow is part of the normal ventral coloration so only a different location of that spot can be considered abnormal. *Pelobates fuscus*, on the other hand, had many yellow spots of various sizes. Unfortunately, we do not have any histological skin sections that would allow a better diagnosis of the described cases.

In 2019, in an artificial pond in the city park Rosarium, Poznań [52°25'26"N, 16°55'56"E], we found free-swimming whitish tadpoles (Gosner stage 25 (Gosner 1960)) of *B. viridis* among several thousands of normally pigmented ones (see the description of this site in Kaczmarski et al. 2019). We caught 10 aberrant and 10 typically colored tadpoles for further rearing. In most atypical individuals, the background color was dirty white or light grey, but the eyes were typically colored (Fig. 2A). During development 6 whitish individuals were affected by edema, curvature of tail and asymmetric bodies. Nine out of 10 reared whitish individuals had trouble freeing their front limbs and finally died before the end of metamorphosis. The color of the individual who survived metamorphosis can be described as leucistic, that is: white, with slightly pigmented spots and typically colored eyes (Fig. 1C, D). All individuals with normal coloration did not show any malformations and underwent metamorphosis without problems.

Bufotes viridis tadpoles typically reach a maximum total length of 52 mm (Günther and Podloucky 1996, according to Henle et al. 2017c), but in our case, three individuals with color aberration reached even 80 mm total length (Fig. 2B), which allows them to be classified as giant (see fig. 17, in Henle et al. (2017c)). As far as we know, gigantism is presumed to be more common among hybrids of *Bufo bufo* (Linnaeus, 1758) and *B. viridis* (Flindt (1984) in Henle et al. (2017c)). However, we confirmed that the here described gigantic tadpoles belonged to *B. viridis* by PCR-amplifying and sequencing

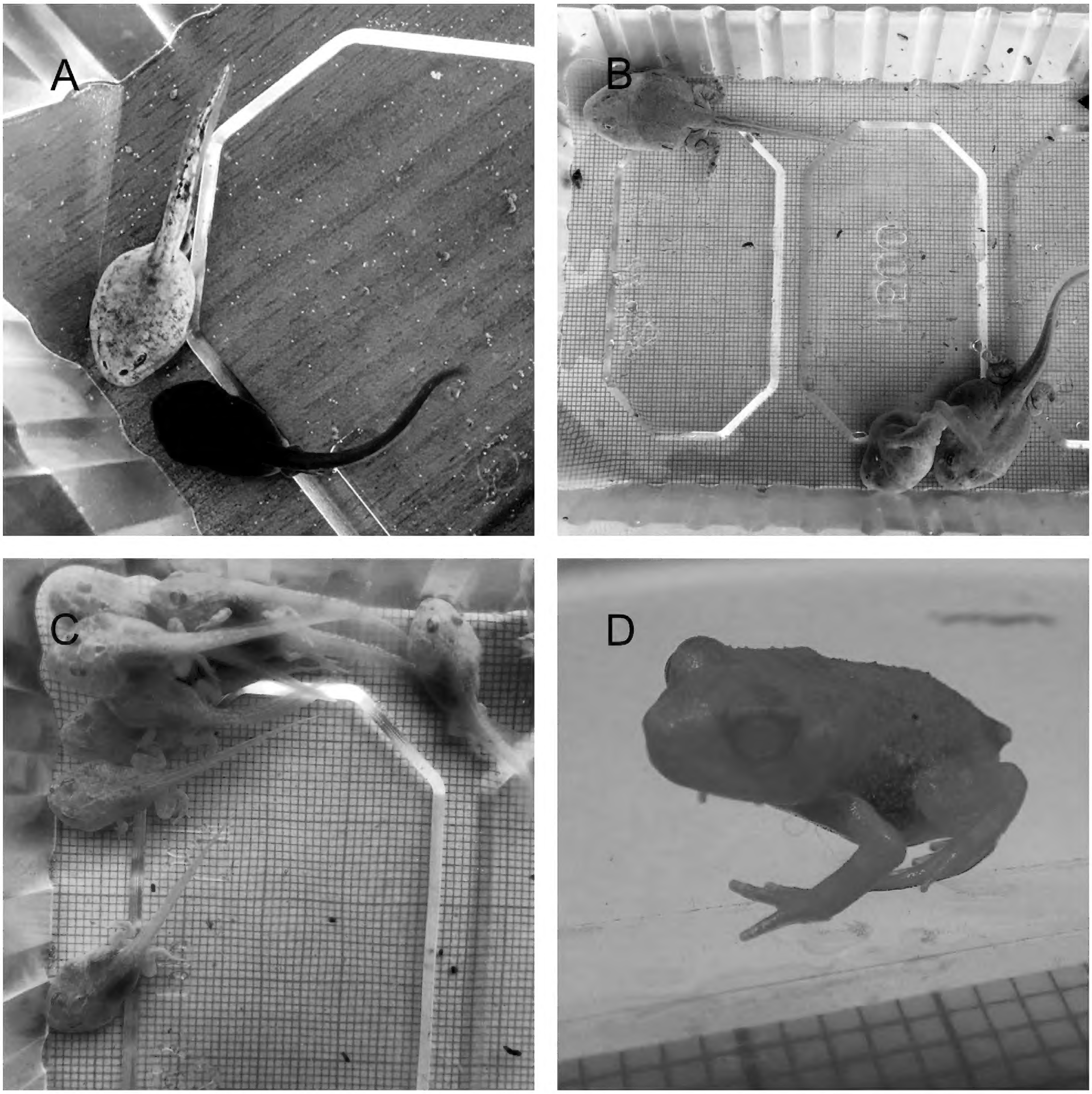

Figure 2. Aberrant tadpoles and juvenile of *B. viridis* from Poland. **A.** Whitish tadpole with a normally pigmented one; **B.** Leucistic giant (52 mm–80 mm **TL**) tadpoles; **C.** Albinistic tadpoles, some of which are undergoing metamorphosis; **D.** Freshly metamorphosed albinistic individual. Eyes are red, which classifies this individual as a complete albino.

one mitochondrial gene (16S rRNA) and one nuclear gene (POMC) and comparing them to reference sequences from *Bufo bufo*, *Epidalea calamita* and *Pseudepidelea viridis/variablis*. Arms remaining within the branchial cavity can be caused by chemical pollution (Henle et al. 2017c). Curvature of tail or body might be related to chemical pollution, inbreeding, increased natural level of UV-B, radioactivity, or other mutagenic factors (Henle et al. 2017c). Gigantism in tadpoles can be induced by chemical pollution or inbreeding (Henle et al. 2017c). Gigantism and leucism in tadpoles of *B. viridis*, along with 30 other anomalies in that species among tadpoles, toadlets, and adults, were described from a quarry near Roßwag (Henle 1981, 1982; Henle et al. 2017c). In our case, the population is located in the city center and occupies a large park area. However, the relatively small number of tadpoles with unusual coloration indicates that the frequency of this mutation does not differ from the norm. Similarly, one leucistic tadpole was recorded in an artificial pond in Milan along with hundreds of normally pigmented conspecifics (Lunghi et al. 2017).

In May 2021, in a temporary pool in an industrial/rural area of Poznań [52°26'24"N, 16°52'53"E], we found 30 free-swimming completely albinistic tadpoles (Gosner stage 25 (Gosner 1960)) of *B. viridis* among several thousands of normally pigmented ones. We caught 10 aberrant and 10 normally colored tadpoles for further rearing. Their development took place without any

problems (Fig. 2C), and the young individuals underwent metamorphosis faultlessly (Fig. 2D). Although albinism in *B. viridis* has already been described several times in the literature (Flindt 1985; Andrä 2011; Lunghi et al. 2017; Strachinis and Tsarouhas 2021), as far as we know, our case is the second one recorded in Poland (Życzyński 2002). Albinism has been associated with spontaneous tyrosinase mutations in three Japanese frog species (Miura et al. 2018). In *B. viridis* color aberrations can be heritable, caused by recessive genes (Boschwitz 1963). They can also be transient; in some cases, the eggs are white, and the hatched tadpoles acquire pigmentation over time (Christaller 1983; Flindt 1985; Henle et al. 2017b). With intensifying anthropogenic pressure, and cameras becoming more accessible and omnipresent, we are likely to see more reports of anomalies in amphibians. However, their causes can be unravelled only with the use of controlled conditions that would allow the measurement of as many potential causal factors as possible.

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